AMENDMENTS TO THE DRAWINGS

Filed herewith are formal drawings. These drawings supersede and replace the drawings filed in the instant application on May 5, 2004.

The formal drawings include changes to Fig. 3. As the Examiner has requested, Fig. 3 has been redone in color using red and green to represent the induced and repressed genes as described in the Brief Description of Drawings.

The drawings are accompanied by a Petition to Accept Color Drawings under 37 C.F.R § 1.84(a)(2) and the fee required under § 1.17(h).

REMARKS

In view of the following remarks, the Examiner is requested to allow claims 1-7, 10, 11, 13, 18, 19, 23, 28, 31 and 33-37, the only claims pending and under examination in this application. Claims 1 and 5 are amended. No new matter is added.

Claim 5 has been amended to be presented in independent form. Claim 1 has been amended to clarify that the reactive moiety present in the purine or pyrimidine analog remains present in said mRNA. Such is evident from the methods utilizing the reactive moiety present on the newly synthesized RNA.

Objections to the Drawings

The drawings have been objected to because Figure 3 is not in color. As indicated above, Figure 3 has been amended. In view of the amendment to Figure 3, this objection may be withdrawn.

Objections to the Specification

The specification has been objected to for lacking appropriate trademark designations. As indicated above, the specification has been amended. In view of the amendment to the specification, this objection may be withdrawn.

Claim Rejections - 35 U.S.C. § 103

Claims 1-7, 10, 11, 13, 18, 19, 23-28 and 31 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Trudeau et al. (Human Gene Therapy 12:1673-1680 (2001)) in view of Johnson et al. (Proc. Natl. Acad. Sci. USA 88:5287-5291(1991)) and further in view of Rana (US Publication 2004/0175732). Applicants respectfully submit that the present claims are not made obvious by the cited art.

According to the M.P.E.P. § 706.02 (j), to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

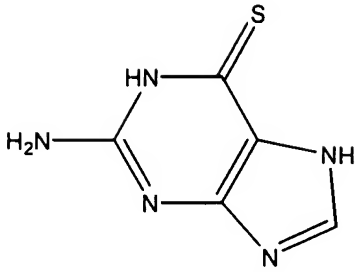
The present invention provides methods for differential biosynthetic labeling of RNA. The label thus introduced is a purine or pyrimidine analog that provides a tag for quantitative separation of the RNA away from unlabelled RNA, or for addition of a second moiety that provides for a detectable label. Using this technique, RNA so labeled can be efficiently and specifically isolated away from all other RNA and analyzed, *e.g.* by hybridization methods such as "Northern" blots and microarray analysis. The RNA thus labeled can be used to quantitate newly synthesized RNA independent of any pre-existing RNA, and can rapidly and sensitively detect changes that occur when genes are switched on or off. The methods of the invention are also useful for purification of specifically labeled RNA. The reactive moiety permits determination of interaction between RNA and proteins, nucleic acids, and other molecules, *e.g.* by cross-linking of the moiety to nearby atoms.

Labeling is performed by using endogenous genes or introduced genetic sequences encoding a phosphoribosyl transferase or nucleoside kinase, which can specifically catalyze the transfer of the purine or pyrimidine analog into the corresponding nucleotide within a cell of interest. The cell of interest is contacted with the purine or pyrimidine analog, which crosses cell membranes and enters the cell. Once converted into the corresponding nucleotide, the analog remains in the cell to be triphosphorylated and incorporated into newly synthesized RNAs, thereby providing for highly selective labeling.

Claim 1 is directed to a method of biosynthetically labeling RNA in a cell of interest. The method includes contacting a cell with a purine or pyrimidine analog. The purine or pyrimidine analog has a reactive moiety not normally present in RNA. As currently amended, the claim has clarified the fact that the reactive moiety is incorporated into RNA by the cell, in order to provide a suitable substrate for the step of obtaining RNA from the cell and conjugating a tag to the reactive moiety that is present on the RNA.

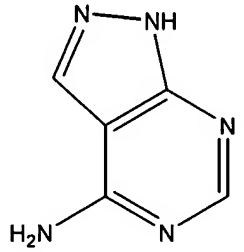
The Examiner has stated that Trudeau teaches contacting a cell with an analog such as thioguanine. Thioguanine is converted to 4-aminopyrazolo pyrimidine ribonucleotide in the cell, and incorporated into parasite mRNA.

Applicants note that Trudeau teaches the introduction of a guanine analog having a structure as shown below, where there is a reactive group at the 6 position.



6-thioguanine

However, an important point is that this reactive group is not incorporated into the parasite mRNA. As stated in the Office Action at page 5, the analog is converted into APPR-MP. The structure for this compound is as follows:



4-aminopyrazolo[3,4-d]pyrimidine

It can be readily observed that the reactive group is no longer present, and thus is not incorporated into the host cell RNA, and cannot provide a reactive moiety not normally present in nucleic acids.

The Office acknowledges that Trudeau is deficient in that it does not teach obtaining RNA from a cell or conjugating a tag to the reactive moiety that is not normally present in the RNA. Applicants submit that such a deficiency goes further, in that the RNA of Trudeau lacks the reactive moiety, and thus could not be conjugated to a tag.

The Office relies on Johnson and Rana to remedy the deficiencies of the primary reference. The Applicants, however respectfully disagree and contend that a *prima facie* case of obviousness has not been established because there is no motivation to combine the references in the manner suggested, and because an essential feature of the biosynthetic labeling method, *i.e.* the label, is not present in the RNA produced by the methods of Trudeau *et al.*

The Office asserts that it would be obvious to modify Trudeau in view of Johnson and Rana because Johnson allegedly discloses that the use of Affi-Gel 501 is inefficient in recovering 4-thiouridine labeled RNA, and Rana allegedly discloses that the use of biotin to tag to thiolated RNA can increase recovery.

The Applicants respectfully disagree and contend that there is no motivation to combine the references in the manner suggested because the proposed modification would change the principle of operation of Trudeau, and indeed would impart to Trudeau a compound that it does not teach - RNA comprising a reactive moiety not normally present in nucleic acids.

Trudeau is not directed to biosynthetically labeling RNA in a cell, but rather to killing a cell (e.g., a lung cancer cell) by sensitizing the cell to allopurinol. The stated use for this method is as a therapeutic tool for gene prodrug targeting of lung cancer. See the Abstract. Hence, Trudeau is not concerned with producing thio-labeled RNA but rather to producing cytotoxic metabolites that induce apoptosis in a cell. Once the cell has been killed, Trudeau has no use for any of the remaining cellular components. Accordingly, Trudeau neither teaches nor suggests either obtaining RNA from the killed cells and/or conjugating a tag to the RNA obtained. One of skill in the art would not be motivated to modify Trudeau such that after the cells are killed RNA from the killed cells is obtained and conjugated with a tag, because to do so, even if possible, would serve no purpose and would therefore be to change Trudeau's principle of operation in contravention of M.P.E.P. § 2143.01.

Applicants have provided herewith an article by Pfefferkorn *et al.* (2001), which describes, similarly to Trudeau *et al.*, the activity of thiolated purine analogs in Roxoplasma gondii. Importantly, the authors find that the analogs are not incorporated into the host DNA, but rather act to inhibit synthesis of guanine nucleotides by blocking IM{-dehydrogenase, thus teaching away from the present invention.

In view of the above, the Applicants contend that a *prima facie* case of obviousness has not been established because one cannot modify the RNA obtained by Trudeau et al because the reactive moiety is not present in the RNA, and thus is not available for conjugation. Further, to make such a modification to Trudeau in the manner suggested changes the principle of its operation. Accordingly, the Applicants respectfully request that the 35 U.S.C. § 103(a) rejection of Claims 1-7, 10, 11, 13, 18, 19, 23-28 and 31 be withdrawn.

Claims 33-35 and 37 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Al-Anouti et al. (Biochemical and Biophysical Research Communications, 302:316-323 (January 2003)) in view of Johnson et al (Proc. Natl. Acad. Sci. USA 88:5287-5291(1991)).

Claim 33 is directed to a method of biosynthetically labeling RNA in a cell of interest. The method includes contacting a cell containing a uracil phosphoribosyltransferase (UPRT) that can convert a uracil analog to a corresponding uridine monophosphate with a uracil analog having a reactive thiol moiety not normally present in RNA, wherein the uridine analog is incorporated into RNA synthesized by the cell.

The Office acknowledges that Al-Anouti is deficient in that it does not teach the incorporation of the uracil analog into RNA synthesized by the cell. The Office, therefore, relies on Johnson to remedy these deficiencies. The Applicants, however respectfully disagree and contend that a *prima facie* case of obviousness has not been established because there is no motivation to combine the references in the manner suggested.

As set forth above, the rejected claims are directed to a method of biosynthetically labeling RNA in a cell. Al-Anouti, on the other hand, is directed to a method of modulating gene expression of *T. gondii* using dsRNA. In accordance with the methods disclosed by Al-Anouti, a *T. gondii* parasite is transfected with a vector containing the genetic sequence for UPRT, from which dsRNA homologous to UPRT is produced. The dsRNA is degraded into small interfering RNA (siRNA) which then interferes with the UPRT gene so as to down-regulate UPRT gene expression, resulting in decreased UPRT activity. See page 323, 2nd full paragraph. Thus, Al-Anouti proposes a method for <u>decreasing</u> UPRT activity, not for utilizing UPRT activity in the biosynthetic labeling of RNA.

The Office, asserts that it would be obvious to modify Al-Anouti in view of Johnson because Johnson allegedly discloses that 4-thiouridine may be used for labeling RNA and that thiolated RNA can provide accurate estimates of transcript half-life. The Applicants respectfully disagree and contend that there is no motivation to combine the references in the manner suggested because the proposed modification would change the principle of operation of Al-Anouti.

According to the M.P.E.P. § 2143.01 if a proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious.

As described above, Al-Anouti is not directed to biosynthetically labeling RNA in a cell, but rather to a method of using dsRNA to modulate cellular expression of genes, with the purpose of making a drug to inhibit the growth of Toxoplasma. See page 323, 2nd full paragraph. Hence, Al-Anouti is not concerned with producing a thio-labeled uridine analog that is incorporated into RNA synthesized by the cell. This can clearly be seen by the fact that Al-Anouti recognizes that the production of 5-fluorodeoxyuridine monophosphate inhibits the synthesis of thymidine monophosphate and is therefore lethal to the parasite. See page 316, 1st paragraph. The only function of such a mechanism with respect to the methods of Al-Anouti is for determining which parasites are expressing the dsRNA that down regulates UPRT gene expression, wherein the parasites that do not contain the siRNA are killed. Accordingly, Al-Anouti neither teaches nor suggests contacting a cell containing a uracil phosphoribosyltransferase with a uracil analog in such a manner that the uridine analog is incorporated into RNA synthesized by the cell.

Therefore, the Applicants contend that given Al-Anouti's purpose of using dsRNA to modulate cellular expression of genes, one of skill in the art would not be motivated to modify Al-Anouti, in view of Johnson, such that instead of modulating the expression of genes, Al-Anouti is producing biosynthetically labeled RNA, because to do so would serve no purpose and would therefore be to change Al-Anouti's principle of operation in contravention of M.P.E.P. § 2143.01.

In view of the above, the Applicants contend that a *prima facie* case of obviousness has not been established because modifying Al-Anouti in the manner suggested changes the principle of its operation. Specifically, instead of being directed to modulating the expression of genes, Al-Anouti would be directed to producing biosynthetically labeled RNA. Consequently, there is no motivation to combine the references in the manner suggested. Accordingly, the Applicants respectfully request that the 35 U.S.C. § 103(a) rejection of Claims 33-35 and 37 be withdrawn.

Claims 36 has been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Al-Anouti et al. (Biochemical and Biophysical Research Communications, 302:316-323 (January 2003)) and Johnson et al. (Proc. Natl. Acad. Sci. USA 88:5287-5291(1991)) as applied to claims 33-35 and 37 above, and further in view of Iltzsch and Tankersley (Biochem. Pharm., 48(4):781-792 (1994)).

Claim 36 depends from Claim 33. The Applicants contend that the combination of Al-Anouti and Johnson is deficient because there is not motivation to combine the references in the manner suggested. As described above, there is not motivation to combine the references because to do so would be to change the principle of operation of the methods of Al-Anouti. As Iltzsch and Tankersley was cited solely for its disclosure of a uracil analog 2,4 dithiouracil, it fails to provide the requisite motivation to combine the references. Accordingly, the Applicants contend that a *prima facie* case of obviousness has not been established because there is no motivation to combine the references in the manner suggested. The Applicants, therefore, respectfully request that this rejection be withdrawn.

CONCLUSION

Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone Dr. Pamela Sherwood at (650) 833-7790.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number STAN-304.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: January 23, 2007

By: _____
Pamela J. Sherwood
Registration No. 36,677

Enclosure(s): New Sheets of Drawings (Fig. 3 only, in triplicate)
Petition to Accept Color Drawings

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